

Comparative behaviour analysis of some colubrids with reference to suitability of captive bred snakes for reintroduction to natural habitat

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Abstract: *Comparative behaviour analysis of some colubrids with reference to suitability of captive bred snakes for reintroduction to natural habitat.* A sample of newly hatched American colubrids, divided according to their level of domestication, understood herewith as the generation history in captivity, was tested in an open field test (OFT) for degree of activity and confidence in open terrain and in confrontation with individuals of such species as *Pantherophis guttatus* and *Lampropeltis getula splendida* as well as *L. g. californiae*, in order to compare their antagonistic and feeding behaviour reactions. The cluster comparison of defence reactions revealed no effect of domestication (many generation in captivity) in the face of potential danger. Even albino phase Kingsnakes, considered to be the form of this species most affected by captivity (altered genome), maintained their natural feeding response. The degree of activity in OFT conditions was seen to decrease with the snakes' age. Thus it was demonstrated that captive specimens bred in terrariums may be considered a gene bank for *ex situ* conservation strategy. However it is recommended that specimens as young as possible should be used in reintroduction to natural habitat, as these will respond most faithfully to the pressure of natural selection, based on their behavioural variation not suppressed by apathy caused by captivity.

Key words: *Lampropeltis*, *Pantherophis*, antagonistic and feeding reactions, captive breeding, *ex situ* conservation

INTRODUCTION

Herpetofauna belongs to that most vulnerable group of animals and those most endangered by anthropogenic pressure to their natural habitat. There have been many attempts of *in situ* conservation and some have proved quite effective – for more information on this, see the Materials of VI World Congress of Herpetology – Manaus 2008. Despite these efforts, more and more trials are undertaken for *ex situ* strategies. Traditionally this activity is the domain of universities or ZOOs, which maintain certain species as gene banks – potential sources of material for reintroduction. In the case of snakes, there are quite a few examples of attempts in this field. During the years 1980–1986, some 34 specimens of captive bred Rocky Python *Python sebae* were released into the Great Fish River Nature Reserve (Eastern Cape), but there was no follow-up (Alexander and Marais 2007, Mattison 2013 – personal communication). In the USA, five AZA facilities have created a consortium “Conservation

Centre for Species Survival” undertaking preparatory work for reintroduction programs for various taxons, including snakes. The species of choice are to be the San Francisco garter snake *Thamnophis sirtalis tetrataenia* and the Louisiana Pine Snake *Pituophis ruthveni* (Mattison 1995, Conway 2011, Mattison 2013 – personal communication). Godwin et al. in 2008 presented a comprehensive report on the captive propagation of the critically endangered Eastern Indigo Snake *Drymarchon couperi*, which included suggestions concerning other snake species. An initial scenario was developed for supplementing the local population of Grey Banded Kingsnake *Lampropeltis alterna* by captive animals (Spanowicz and Życzyński 2002). Similar efforts were undertaken by Łódź University, in cooperation with Łódź ZOO, for a domestic (Polish) species – Smooth Snake *Coronella austriaca* (Zieliński and Stanisławski 2001, Stanisławski 2003). All these attempts led to the release of captive snakes into the wild, but were not continued after the first trial.

Another attempt worth mentioning was the successful reintroduction of the Antiguan Racer *Alsophis antiquae* (Daltry 1999 and 2006). In this project, the reintroduced snakes were field collected on Great Bird Island, tagged and released on small Rabbit Island. On the contrary the results of reintroduction of Woma Python *Aspidites ramsayi* in Arid Recovery Reserve in northern South Australia did not meet the short-term and medium-term success criteria mostly due to predator pressure (Moseby et al. 2011).

Potential support for such work may come from private enthusiasts and keepers. This is a significant new idea. Al-

though as early as 1975, John Coborn organized the symposium “Conservation and Captive Reptiles and Amphibians”, where the case was made by many herpetologists for and against captivity as an aid to the preservation of wild species, there is still much work to be done in this field. In any case, it must be admitted that the level of knowledge of herpetofauna husbandry is constantly growing and additionally, the pure breeding strategy according to locality of origin has become the ethical rule among the most advanced keepers. As a result, in many cases the numbers of certain species in captivity exceed those ever to be seen in the wild and these can be treated as pure gene banks. Their active population, with a quite large effective number (N_e) is much greater than could be assured by official institutions limited by state budget conditions. But if we are to treat captive animals as candidates for restocking, we must be sure that these fulfil the demands of gene bank purity, as well as the demands with regard to sanitarian status, in order to prevent introduction of exotic pathogens to the natural habitat. The captive material must also exhibit the natural degree of fitness that can be subjected to natural selection.

The aim of the present study is a comparative behavioural analysis of captive bred snakes, which can be differentiated according to the level of their domestication (understood herewith as the multi-generation history in terrariums’ life).

MATERIALS AND METHODS

The snakes used in this study were born in Laboratory of Department of Genetics and Animal Breeding of WULS

– SGGW. They belonged to the genera *Lampropeltis* and *Pantherophis*. Authors chose these taxons in view of their popularity among keepers. Compared to other genera, most of these snakes have a quite long generation history in captivity and thus constitute interesting material for an evaluation of the level of natural behaviour preserved. These may serve as a model for conclusions concerning other, potentially truly endangered species, which are currently not stocked in collections.

The material was divided into the following groups:

1. *Ls* × *Lc* – inter subspecies cross between Sinaloan Milk Snake *Lampropeltis triangulum sinaloe* and Pueblan Milk Snake *Lampropeltis triangulum campbelli* – (parents from multigenerational captive breeding) – here – captive (domesticated) type – 10 specimens;
2. *Lpp* × *Lpk* – inter subspecies cross between Arizona Mountain Kingsnake *Lampropeltis pyromelana pyromelana* and Chihuahua Mountain Kingsnake *Lampropeltis pyromelana knoblochi* – second generation in captivity – intermediate type – 3 specimens;
3. *Ege* – Great Plains Rat Snake *Pantherophis emoryi* – from field collected parents – wild type – 8 specimens;
4. *Ege* × *Egg* – interspecies cross between Great Plain Rat Snake *Pantherophis emoryi* (field collected) and Corn Snake *Pantherophis guttatus* (amelanistic form – fixed mutation in multigenerational captivity) – intermediate type – 6 specimens;
5. *Eggalb* × *Eggan* – *Pantherophis guttatus* – a crossbreed of two fixed mutations – snow albinism and anerytristic (black albinism) – “domestic” type – 7 specimens.

The snakes were subjected to open field test – OFT (Markowska 1979) scoring points for movement activity within the field. Animals were placed within a ring 90 × 90 cm, fenced with wooden walls. The surface of the ring consisted of squares 30 × 30 cm forming three rows. The scores for activity were counted as follows: snake was getting one point when slithered along one square adjacent to the walls and 3 points for distancing from the ring walls and entering the square in the centre of the field. After 5 min of observation the points were added up.

Later the snakes were confronted with a young Corn Snake *Pantherophis guttatus* – a species preferring rodents as the natural diet, and a Desert Kingsnake *Lampropeltis getula splendida* – living (as the whole genus *Lampropeltis*) on lizards and other snakes as its most typical food. The points scored and body weights of each snake tested were subjected to one-way ANOVA. When necessary the F-test was followed by D-test to verify the significance of differences among the groups. Additional analysis of covariance with body weight as an operand variable was done to check whether the sizes of snakes could affect their confidence and thus the results of OFT test.

During confrontations (lasting 5 min each) with another snake, the following reactions were noted on a Yes or No basis: lack of any reaction, strong tail rattling (loud and lasting longer than 5 s), weak tail rattling (lasting less than 5 s), tossing movements, sudden retreat, avoidance and attack.

In authors' experience, the chosen/described reactions reflect well the emotional status of the snake during the test. The probabilities of each reaction in two

separate trials, for confrontations with Corn Snake *P. guttata* and Desert Kingsnake *L. g. splendida* and combined, were subjected to cluster analysis (Nei 1972) permitting to build dendrograms of behavioural distance.

In an additional analysis (not included in all experiments), authors used Sinaloan Milk Snake *Lampropeltis triangulum sinaloe* – 4 specimens, Grey-banded Kingsnake *Lampropeltis alterna* – 2 specimens, and California Kingsnake *Lampropeltis getula californiae* (albino) – 2 specimens. All of these were born a year later. These snakes were confronted with each other within their own species and with albino Kingsnakes.

RESULTS

The points scored in OFT in both observations met the criteria of normal distribution with mean values 7.7 and standard deviation 5.4 and mean value 8.5 with standard deviation 5.5 for I and II trial respectively.

The differences between groups are significant $P \leq 0.05$ for the I observation

only. As the snakes aged, the differences between groups disappeared.

The score in the II observation is generally smaller – *vide* Table 1. This can be explained by increased apathy caused by captive conditions.

The ANOVA results for snakes body weights were significant $P \leq 0.05$. As the activity in OFT could be affected by snakes' sizes we made the additional analyses, correcting the analysis for points scored in the first observation by deviation from regression line for body weights.

The deviations from regression line (body weight as an operand variable) are highly significant ($P \leq 0.01$) and confirm that the significant differences ($P \leq 0.05$) in points scored in OFT (I observation – Table 1), were not caused by differences in the sizes of specimens, but in reality depended on group classification.

Activity in the field test was lower during the II observation except in the case of the group 5. Growth was different in each group – in the *pyromelana* group, this was even a negative value.

The results for cluster analysis are shown in the dendrograms in Figures 1, 2, 3, 4 and 5.

TABLE 1. Points scored in OFT and measurements of body weights during the I and II observations

Groups	N	Scores in OFT		Body weights [g]	
		I Observation	II Observation	1st Observation	2nd Observation
		$\bar{x} \pm \sigma$	$\bar{x} \pm \sigma$	$\bar{x} \pm \sigma$	$\bar{x} \pm \sigma$
<i>Ls</i> × <i>Lc</i>	10	7.9 ± 0.9 ^a	7.3 ± 3.2	35.2 ± 3.1 ^{A;E}	36.4 ± 3.3
<i>Lpp</i> × <i>Lpk</i>	3	3.3 ± 1.8 ^{b;d}	1.7 ± 2.1	27.7 ± 1.0 ^{B;F}	26.3 ± 3.3
<i>Ege</i>	8	10.9 ± 2.5 ^{d;e}	8.0 ± 5.4	174.9 ± 34.7 ^{A;B;C;D}	185.1 ± 37.0
<i>Ege</i> × <i>Eggam</i>	6	13.2 ± 1.9 ^{a;b;c}	10.3 ± 6.4	112.0 ± 21.1 ^{C;E;F;G;H}	141.9 ± 21.3
<i>Eggan</i> × <i>Eggalb</i>	7	4.7 ± 1.3 ^{c;e}	7.6 ± 1.9	40.5 ± 2.9 ^{D;H}	47.9 ± 4.0

Letters (in pairs): a–e point out the significant differences between means of groups for points scored during the first trial – Duncan test; $P < 0.05$;

Letters (in pairs): A–H show the significant differences between means of groups for body weights during the first trial – Duncan test; $P < 0.05$.

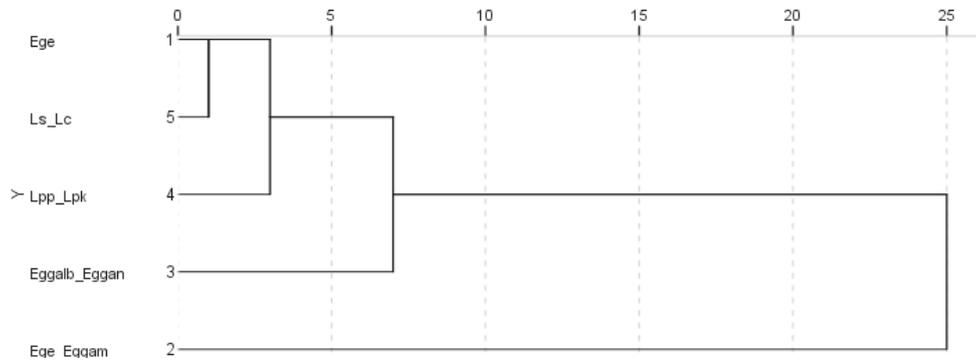


FIGURE 1. Comparison of group reactions during the I observation – confrontation with *Pantherophis guttatus*

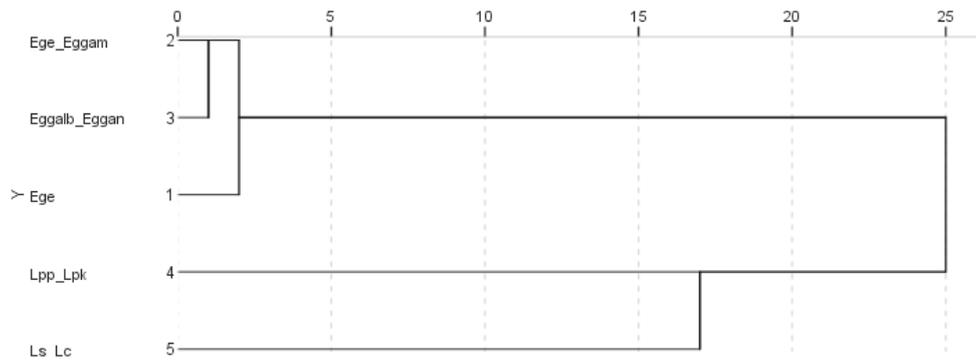


FIGURE 2. Comparison of group reactions during the II observation – confrontation with *Pantherophis guttatus*

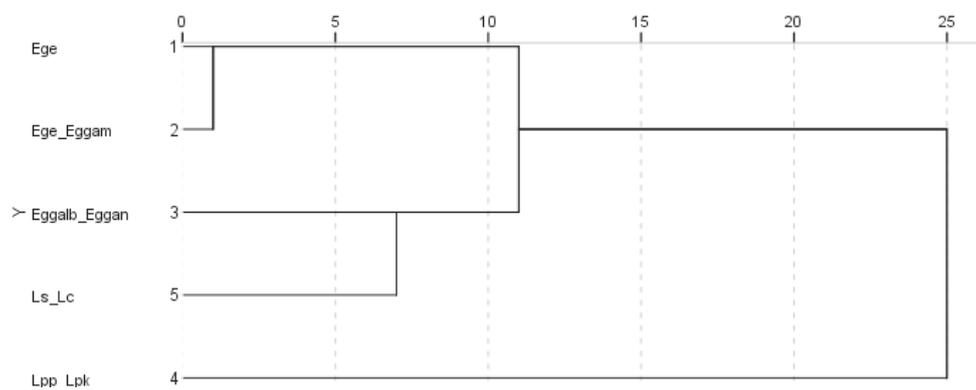


FIGURE 3. Comparison of group reactions during the I observation – confrontation with *Lampropeltis getula splendida*

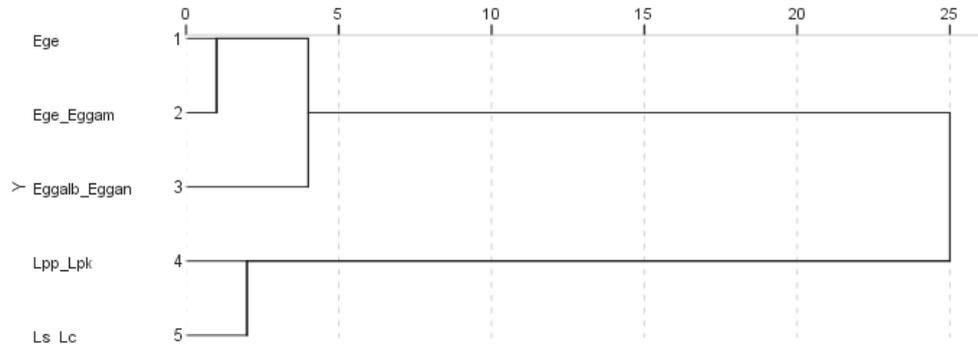


FIGURE 4. Comparison of group reactions during the II observation – confrontation with *Lampropeltis getula splendida*

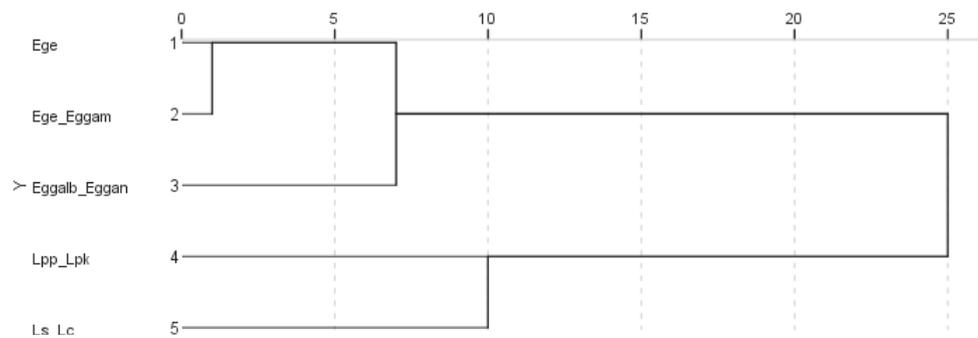


FIGURE 5. Comparison of group reactions – both types of confrontation grouped together

Confrontation with the relatively harmless Corn Snake *Pantherophis guttatus* has not revealed any differences in reactions between groups, which could be attributed to either the level of domestication or the taxonomic position.

In contrast to the I observation, during the II observation, taxonomy played an important part in sorting the snake groups within clusters. This tendency remained constant during subsequent trials – *vide* Figures 3–5.

Figures 3–5 show dendrograms that are practically identical with Figure 2. There is no noticeable influence of domestication or the addition of “wild

blood” as a factor differentiating the groups.

During confrontation with their own species (Greybanded Kingsnakes, Milk Snakes and California Kingsnakes), authors observed some slight excitation (avoidance, tail rattling) in the tested snakes. Confrontation with the albino Kingsnake resulted in an attack by the latter showing the typical feeding response. The Kingsnakes themselves did not react to each other.

Following the attack, the trials were immediately halted, with no harm being caused to the snakes by the testing procedure.

DISCUSSION

Analysis of the snakes' activity in OFT showed significant differences only during the I observation (Table 1). Comparison within genus *Pantherophis* revealed that groups 3 and 4 did not differ significantly from each other but both deviate from group 5 (the smallest specimen – Table 1) designated the most domesticated – the longest generation history in captivity. Correction on regression line for body weight showed that points scored in OFT do not depend on body weight. This result accords with the fact that during the II observation, differences between groups in terms of body weight increased, but differences in activity (points scored in OFT) disappeared (*vide* Table 1).

Based on these findings, we can conclude that snakes kept for generations in captivity behave less actively than those field collected or those with the addition of "wild blood". We may ask whether decreased activity reflexes lower fitness ability. Each group of reptiles has its own survival strategy. Research carried on turtles (Mrosovsky and Gogfrey 1995) has shown that the more active young (1YO) specimens have a lower survival rate than those less mobile, whereas the higher mobility of young lizards (Van Damme et al. 1992) provides them a greater success rate in hunting and a greater ability to avoid predators. If greater activity and courage in young snakes protects them in the wild, researchers may conclude that a longer generation stage in captivity debilitates adaptation and fitness. This is true for the first stages of life only, as during the II observation all groups behaved similarly

– probably due to apathy caused by captive conditions.

Another source of information is provided by analysis of the dendrograms. The I confrontation, with the relatively harmless Corn Snake (Fig. 1), showed a considerable variance in reactions between groups, which could not be attributed to either taxonomy or level of domestication. The II confrontation, with a Corn Snake and the effect of exposing the tested specimens to more dangerous stimuli i.e. Desert Kingsnake *L. g. splendida*, (Fig. 2–5) provided uniform dendrograms arranged according to taxonomy and not the captivity status.

Albino Kingsnakes tolerated each other well, but in the presence of another species, manifested an immediate feeding response by attempting to attack.

Rodriguez-Robles and De Jesus-Escobar (1999) studied Colubridae evolutionary relationships by analyzing the mt DNA variability and adjusting their conclusions to the evolution of feeding customs. According to the authors, a diet based on reptiles (lizards, snakes) is more primitive than one based on rodents, which developed much later. They presented their results in the form of dendrograms, showing both the genetic relations between genera of American colubrids and the hypothetic evolution of feeding behaviour.

The results presented earlier correspond with those quoted. Divergence in behaviour during confrontations with predators phylogenetically approximates feeding behaviour and differs by definition from inner aggression.

The divergences noted above are very important from the point of view defined in the title of this study. During the first

trials, we observed diversity in both the fitness of the specimens and their reactions during confrontation. These differences were reduced as the snakes got older. So any decision to reintroduce snakes should only consider very young specimens, i.e. hatchlings. Such material may be characterized by greater fitness – *vide* Table 1, although due to their smaller size, these could be more at risk from predators. But this would allow for natural selection, a factor always present in the natural habitat.

The accordance of reactions with the dendrograms shown by Rodriguez-Robles and De Jesus-Escobar revealed that captive *Lampropeltis* snakes preserved their natural feeding behaviour. Thanks to their undisputed beauty, Kingsnakes and Milk Snakes have gained enormous popularity among amateur snake keepers. In captive conditions, they are forced to adapt to a rodent based diet. Some specimens adapt to this easily; others are more reluctant. Nevertheless their maintenance in captivity is increasingly easy and the survival rate of hatchlings is gradually increasing. This fact is worthy of attention. The most popular terrarium species may have been subjected to unintentional selection for the ability to adapt to a diet that is atypical for them. Arnold (1981), working on garter snakes *Thamnophis elegans* proved that feeding preferences are genetically polymorphic and of a quantitative nature. Thus selection for diet type could be possible and may also result in inherited changes in feeding customs, not caused by current artificial captive conditions only. This problem is discussed by Conway 2011, focusing on the number of generations in

captivity as a function of potential, undesirable selection.

Snakes play an important role in the trophic chain. In the event of reintroduction of captive material into the natural habitat, the consequences of releasing animals of genetically changed feeding customs could be at the least undesirable, even where the rules of taxonomic and population fidelity have been obeyed. It is worth mentioning that the team working on the reintroduction of the Smooth Snake *Coronella austriaca* (Zieliński and Stanisławski 2001) were offering their captive hatched snakes live small lizards (*Lacerta agilis*), which were preyed on. This reflected in terrarium conditions the rules of natural selection for the snakes' survival. However, the results of this restocking are difficult to evaluate, as the snakes were not tagged for telemetric monitoring as they were in Daltry's (1999) project.

Lack of proper documentation is always the weakest point of any reintroduction project, as was pointed out by Earnhardt (1999). This particularly concerns survival rates in the very first period of release.

The dendrograms and observations presented in this paper lead to conclusion that studied *Lampropeltis* snakes, despite their history of captivity, preserved their natural feeding habits, enabling them to play their natural role in the trophic chain when returned to their natural environment. Likewise the *Pantherophis* snakes, despite their level of domestication, did not differ from one another in the general set of reactions.

CONCLUSIONS

This model experiment shows that captive snakes from multigeneration terrarium colonies can be considered worthy material for reintroduction, on condition that the principles of genetic fidelity are observed.

A planned release should be carried out at a very young age, despite the expected losses of very small and fragile animals, in order to let them undergo a process of natural selection based on fully exposed diversity.

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Streszczenie: Porównawcza analiza zachowań węży z rodziny Colubridae pod kątem przydatności osobników hodowanych w niewoli do reintrodukcji do środowiska naturalnego. W modelowym doświadczeniu przetestowano amerykańskie węże z rodziny Colubridae podzielone według stopnia udomowienia (pokoleniowego stażu w warunkach niewoli). Zwierzęta zostały poddane testowi otwartego pola (OFT), sprawdzającego ich aktywność i odwagę na otwartej przestrzeni oraz konfrontacji z innym wężem zbożowym *Pantherophis guttatus* oraz lancetogłowem królewskim *Lampropeltis getula splendida* i dodatkowo *L.g. californiae*, formą albinotyczną. Test OFT wykazał ujemny wpływ wieku na aktywność terraryjnych zwierząt a konfrontacje z probantami nie ujawniły wpływu udomowienia na reakcje obronne (klasterowa analiza skupień). Nawet u albinotycznych lancetogłówów, uznanych za najbardziej udomowioną formę (zubożały, wsobny genotyp) utrzymywanych od pokoleń na nie-

naturalnej diecie, wystąpiły normalne reakcje drapieżnicze. Stwierdzono zatem przydatność populacji terraryjnej jako ewentualnego banku genów dla strategii ochronnej *ex situ*, przy zaleceniu używania do reintrodukcji materiału możliwie młodego, najwierniej reagującego na presję selekcji naturalnej, wymierzonej w wachlarz reakcji niestłumionych warunkami niewoli.

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